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Subject: RE: Bloomberg: Roundup Herbicide Hurts Beneficial Bacteria in Bees, Study Finds (1)

In response to a request from the OCSPP-IO, EFED has completed a cursory look at the study and offers the following. I would draw your attention to the last couple of lines in the discussion: “[C]olony-level studies have been submitted for glyphosate and these studies do not indicate impaired colony performance at similar exposure levels. Therefore, the extent to which the effects observed on individual bees under laboratory conditions translate to meaningful impacts at the colony level is uncertain.”

Motta, E. V. S, K. Raymann, N. Moran. 2018. Glyphosate perturbs the gut microbiota of honey bees. Proc. National Acad. of Science. www.pnas.org/cgi/doi/10.1073/pnas.1803880115

Methods: Marked adult worker honey bees (*Apis mellifera*) were fed untreated sucrose solution or glyphosate at 5 or 10 mg/L for 5 days then returned to their colony. Bees (15) were sampled before and 3 days after reintroduction to the colony. DNA was extracted from the gut for quantitative polymerase chain reaction (qPCR) analysis.

Additionally, capped pupae (older-stage pupae) were removed from brood frames and allowed to emerge (referred to as newly emerged workers; NEWs) were exposed to bee gut homogenate for 5 day and then fed 1 mM glyphosate or sugar water on two alternate days; bees were then sampled 2 days after the last feeding and then sampled for gut DNA qPCR analysis. NEWs were also divided into two subgroups and treated with either 0.1 mM glyphosate or sucrose for 5 days and half of the group exposed to the “opportunistic pathogen *Serratia marcescens* kz19” while the other half were not exposed to the pathogen. According to the paper, bees were exposed to similar amounts of glyphosate (~1.7 µg) in the study (based on feeding 5 µL of 1 mM glyphosate).

Results: According to the study authors, the relative and absolute abundances of dominant gut microbiota decreased in bees exposed to glyphosate at environmentally relevant concentrations and glyphosate exposure of NEWs increased mortality of bees subsequently exposed to *S. marcescens*. Gut microbes varied in susceptibility to glyphosate corresponding to whether they have genes belonging to 5-enolpyruvylshikimate-3-phosphate synthase (EDSP; involved in the synthesis of aromatic amino acids), which glyphosate is known to inhibit of class I (sensitive to glyphosate; *Snodgrassella alvi*) or class II (insensitive to glyphosate).

EFED Reviewer Comments: The research has some major uncertainties that could limit its utility to EFED. These uncertainties include:

- Article does not identify whether they tested TGAI or TEP. With glyphosate, this can make a big difference as inert in TEP can markedly affect the activity of the product.
- Only two glyphosate treatment levels were tested (5 and 10 mg/L).
- The graphics in the article are a little difficult to discern; however, plotting gut flora on a per bee basis may be of questionable utility in determining whether there are meaningful differences. The graphics indicate though that although two glyphosate treatment levels were tested (5 and 10 mg/L); there was little to no response in the higher treatment.
- There is no analytical verification of exposure concentrations.
- Most of the residue data reported for various matrices (*e.g.*, pollen, nectar, wax) associated with bee colonies are based on multi-residue (broad spectrum analyses), but it is EFED’s understanding that for these matrices, glyphosate requires a single analyte approach; therefore, the relevancy of the exposure levels is

uncertain. However, in a semi-field colony-level study with a foliar application of a glyphosate TEP (2.56 lb a.e./A) (Thompson, 2014), the mean measured glyphosate exposure levels in larvae 4 days after treatment was 11.9 mg a.e./kg, which may be similar to the concentrations tested in this study. Although, there is uncertainty in this given the uncertainty in the form of glyphosate tested and that they did not verify exposure. In the semi-field study, there did not appear to be impacts to the colony following the glyphosate exposure.

- It is uncertain if the pathogen is environmentally relevant. Not familiar with the “opportunistic pathogen” *S. marcescens* used to challenge the bees. Have not seen this listed as a common pathogen in bees and question whether the bees may have been naive to the pathogen.
- When discussing the ages of bees used in the initial part of the study, the authors were not particularly clear as to the exact age of adult worker. The gut flora of bees is conferred on newly emerged bees by in-hive bees (nurse bees) which feed the developing bees via trophallaxis). The gut homogenate fed to bees in the second part of the study with NEWs intended to confer workers with common microflora; however, this was not done in the first part of the study.
- Work conducted by USDA Agricultural Research Service has indicated that certain fungicides can affect gut microflora and indirectly affect bee nutrition; but haven’t seen similar effects noted for herbicides.

Without more detailed information on the actual test substance, the study would not meet established OPP criteria for use of open literature. The relationship of the study’s measurement endpoints to EFED assessment endpoints is uncertain; while mortality increased in glyphosate treated bees exposed to *S. marcescens* it is uncertain whether immune response of bees would be similarly affected given that newly emerged workers are likely receiving food from nurse bees through a variety of sources as bees tend to forage in a wide range of habitats.

While the study provides some interesting data, colony-level studies have been submitted for glyphosate and these studies do not indicate impaired colony performance at similar exposure levels. Therefore, the extent to which the effects observed on individual bees under laboratory conditions translate to meaningful impacts at the colony level is uncertain.

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Subject: Bloomberg: Roundup Herbicide Hurts Beneficial Bacteria in Bees, Study Finds (1)

For your awareness.

https://news.bloombergenvironment.com/environment-and-energy/roundup-herbicide-hurts-beneficial-bacteria-in-bees-study-finds-1?usertype=External&bwid=00000165-fdd1-d667-a56f-ffdbf86b0002&qid=5450136&cti=FG2&uc=93&et=CURATED_HIGHLIGHTS&emc=neve_hlt%3A4&context=email&email=0000166-0d2c-d885-a5f7-9ffe53970001&access-ticket=eyJjdHh0IjoITkVWRSlmImlkIjoIMDAwMDAxNjUtZmRkMS1kNjY3LWE1NmYtZmZkYmY4NmIwMDAyIiwic2lnIjoITXlqK1A3NUUp3dW5KNXhIQlITQjRIQlVHThAwPSIsInRpbWUiOiIxNTM3ODE5NTk3IiwidXVpZCI6ImNsd3pGRExLTVdwejhheFhDS0pCSkE9PUUxQjBEeWpxam8vYTJEQUJITXBzRFE9PSIsInYiOiIxIn0%3D

Roundup Herbicide Hurts Beneficial Bacteria in Bees, Study Finds (1)

By Tiffany Stecker

- Study shows glyphosate kills gut bacteria in bees
- Adds to body of science showing bacteria's effects on health

A new scientific study could breathe life into the argument that the herbicide Roundup harms animals, insects, and humans by disrupting bacteria in the gut.

Scientists at the University of Texas at Austin unveiled research Sept. 24 finding that honeybees become more susceptible to disease, and eventually die, after their gut bacteria was exposed to glyphosate, the main ingredient in Bayer AG's Roundup herbicide.

Farmers and researchers' concerns about bees have grown since 2007 when scientists began noticing that bee colonies were collapsing.

Gut Check

The new research joins a body of scientific inquiry into how the gut microbiome regulates health in humans, animals, and now bees. The issue also has been used by lawyers to challenge Monsanto Co.—which Bayer bought June 7—on its claim that “glyphosate targets an enzyme found in plants but not people or pets.”

The scientists at the University of Texas treated hundreds of adult worker honeybees with two different concentrations of glyphosate for five days and then released the bees back to their hives. By the third day, the abundance of four dominant gut bacteria were decreased.

The researchers then studied the vulnerability of glyphosate-treated bees to a harmful bacteria. For bees lacking the gut microbes, the exposure to the pathogen increased mortality relative to bees with normal gut bacteria.

“When you perturb these microbial communities you can make them more susceptible to pathogens,” Erick Motta, a graduate student in the laboratory and lead author of the paper, told Bloomberg Environment.

A spokeswoman for Bayer Crop Science rejected the findings.

“Claims that glyphosate has a negative impact on honey bees are simply not true,” Bayer communications representative Charla Lord told Bloomberg Environment. “More than 40 years of robust, independent, scientific evidence shows that it poses no unreasonable risk for humans, animals, and the environment generally.”

‘Unexpected and Expected at the Same Time’

Lord pointed to a 2014 study that found no significant adverse effects from glyphosate on mortality, bee development, or mean weight of bee pupae. She also cited a bee study conducted as part of the European Union's renewal of glyphosate's approval last year.

Glyphosate is the most commonly used herbicide in the world. It kills weeds by inhibiting an enzyme essential for keeping plants alive. The chemical disrupts the pathway that the enzyme—5-enolpyruvylshikimate-3-phosphate (EPSPS)—takes to process amino acids, the building blocks of proteins.

Animals don't process amino acids through the same pathway as plants. But bacteria—including the microbes that populate mammals' intestines—do.

Motta acknowledges that several scientific papers have shown that glyphosate is innocuous to bees. But the indirect effect of glyphosate has not been studied as extensively.

Studies on the effects of antibiotics on bees show that these treatments can compromise the microbial community inside bees. A 2017 [study](#) found that antibiotic treatments—which are used by beekeepers to stave off infections—can affect both the size and composition of the honeybee gut microbiome.

“It's something that's kind of unexpected and expected at the same time,” Motta said.

Global concern over honeybees has grown since 2007, when scientists began documenting a mysterious phenomenon called colony collapse disorder, in which bees flee their hive and die. Pesticides—particularly a class of insecticides called neonicotinoids—have been implicated as a contributing factor in the decline of honeybees. From April 2017 to April 2018, about 40 percent of managed honeybee colonies were lost, according to the federally backed bee [survey](#).

“You can't explain colony collapse disorder with glyphosate,” Reed Johnson, an associate professor of entomology at the Ohio State University who was not involved in the study, told Bloomberg Environment. “But it's one more straw, one more thing making the bees susceptible to disease.”

Judge Says Label ‘Literally False’

Glyphosate has drawn attention from scientists and government agencies for the last three decades, particularly because it complements the adoption of genetically engineered crops that can tolerate applications of the weedkiller.

Regulatory agencies around the world—including the Environmental Protection Agency and the European Food Safety Authority—have said the chemical doesn't cause cancer.

But in 2015 the International Agency for Research on Cancer concluded that glyphosate is a “probable” carcinogen, sparking lawsuits against Monsanto. A jury ordered the company Aug. 10 to pay \$289 million to a California groundskeeper who claimed Roundup caused his non-Hodgkin lymphoma, a type of blood cancer.

“It is an important study in the arena of adverse health effects on pollinators and mammals, as the mechanism is the same—microbiota are an important element of organism's immune systems,” Michael Baum, an attorney with Baum Hedlund Aristei Goldman PC in Los Angeles who is involved in several glyphosate-related lawsuits, told Bloomberg Environment.

A federal court in Wisconsin agreed in April to hear a case challenging the Bayer/Monsanto label on Roundup stating that it doesn't harm people or pets.

The judge in *Blitz v. Monsanto Co.* concluded that a “reasonable” consumer would take the statement that “glyphosate targets an enzyme found in plants but not people or pets” to mean that the enzyme is not present in people, rather than not found in human cells.

“Many consumers might well think gut bacteria are located *in* people. Under that view, the Roundup label would again be literally false,” U.S. District Court for the Western District of Wisconsin Judge William M. Conley wrote in his opinion.

Similar “enzymatic pathway” arguments against Monsanto have failed in the past. Federal courts in New York and California dismissed cases when plaintiffs sought relief in the form of court-ordered label changes.

The cases were thrown out because one of the nation’s pesticide laws—the Federal Insecticide, Fungicide, and Rodenticide Act—dictates what information makes it onto pesticide labels and pre-empts any injunctive relief claims.

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